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# Propofol-based anaesthesia versus sevoflurane-based anaesthesia for living donor kidney transplantation: results of the VAPOR-1 randomized controlled trial

G. J. Nieuwenhuijs-Moeke<sup>1,\*</sup>, V. B. Nieuwenhuijs<sup>2,3</sup>, M. A. J. Seelen<sup>4</sup>, S. P. Berger<sup>4</sup>, M. C. van den Heuvel<sup>5</sup>, J. G. M. Burgerhof<sup>6</sup>, P. J. Ottens<sup>2</sup>, R. J. Ploeg<sup>2,7</sup>, H. G. D. Leuvenink<sup>2</sup> and M. M. R. F. Struys<sup>1,8</sup>

<sup>1</sup>Department of Anaesthesiology, <sup>2</sup>Department of Surgery, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands, <sup>3</sup>Department of Surgery, Isala, Zwolle, The Netherlands, <sup>4</sup>Department of Nephrology, <sup>5</sup>Department of Pathology, <sup>6</sup>Department of Epidemiology, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands, <sup>7</sup>Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK and <sup>8</sup>Department of Anaesthesia, Ghent University, Ghent, Belgium

\*Corresponding author. E-mail: g.j.nieuwenhuijs-moeke@umcg.nl

## Abstract

**Background.** Kidney transplantation is associated with harmful processes affecting the viability of the graft. One of these processes is associated with the phenomenon of ischaemia-reperfusion injury. Anaesthetic conditioning is a widely described strategy to attenuate ischaemia-reperfusion injury. We therefore conducted the Volatile Anaesthetic Protection of Renal Transplants-1 trial, a pilot project evaluating the influence of two anaesthetic regimens, propofol- vs sevoflurane-based anaesthesia, on biochemical and clinical outcomes in living donor kidney transplantation.

**Methods.** Sixty couples were randomly assigned to the following three groups: PROP (donor and recipient propofol), SEVO (donor and recipient sevoflurane), and PROSE (donor propofol and recipient sevoflurane). The primary outcome was renal injury reflected by urinary biomarkers. The follow-up period was 2 yr.

**Results.** Three couples were excluded, leaving 57 couples for analysis. Concentrations of kidney injury molecule-1 (KIM-1), N-acetyl- $\beta$ -D-glucosaminidase (NAG), and heart-type fatty acid binding protein (H-FABP) in the first urine upon reperfusion showed no differences. On day 2, KIM-1 concentrations were higher in SEVO [952.8 (interquartile range 311.8–1893.0) pg mmol<sup>-1</sup>] compared with PROP [301.2 (202.0–504.7) pg mmol<sup>-1</sup>]. This was the same for NAG: SEVO, 1.835 (1.162–2.457) IU mmol<sup>-1</sup> vs PROP, 1.078 (0.819–1.713) IU mmol<sup>-1</sup>. Concentrations of H-FABP showed no differences. Measured glomerular filtration rate at 3, 6, and 12 months showed no difference. After 2 yr, there was a difference in the acute rejection rate ( $P=0.039$ ). Post hoc testing revealed a difference between PROP (35%) and PROSE (5%;  $P=0.020$ ). The difference between PROP and SEVO (11%) was not significant ( $P=0.110$ ).

**Conclusions.** The SEVO group showed higher urinary KIM-1 and NAG concentrations in living donor kidney transplantation on the second day after transplantation. This was not reflected in inferior graft outcome.

**Clinical trial registration.** NCT01248871.

**Key words:** biomarkers; kidney transplantation; propofol; reperfusion injury; sevoflurane

**Editor's key points**

- Ischaemia reperfusion injury (IRI) may affect outcome after several types of surgery including kidney transplantation
- Anaesthetic agents may attenuate IRI to varying degrees through preconditioning, but the effect on outcome after kidney transplantation is unknown
- In this randomized study, there were some differences in early urinary biomarkers of kidney injury between patients receiving Sevoflurane or propofol-based anaesthesia
- There were no significant differences in outcome between groups, but the study may have been underpowered to detect this

Anaesthetic conditioning (AC) is the ability of anaesthetic agents to induce biochemical changes that may attenuate ischaemia-reperfusion injury (IRI).<sup>1</sup> These capacities are attributed in particular to volatile anaesthetic (VA) agents, such as sevoflurane or isoflurane, and to a much lesser extent to propofol. Depending on the timing of administration, it is defined as preconditioning (before ischaemia), perconditioning (during ischaemia), or postconditioning (directly upon reperfusion). Protective effects of AC of VA on the heart are demonstrated *in vitro*, in animal species, and in randomized controlled clinical trials.<sup>2–4</sup> In contrast, in kidneys the evidence for AC of VA is restricted to *in vitro* and animal work. Rats anaesthetized with VA and subjected to renal IRI showed reduced concentrations of plasma creatinine and cytokines, reduced pro-inflammatory leucocyte infiltration, and reduced histological renal necrosis compared with rats anaesthetized with pentobarbital or ketamine.<sup>5</sup> In mice, anaesthesia with isoflurane led to reductions of neutrophil, macrophage, and lymphocyte infiltration after renal IRI compared with pentobarbital anaesthesia.<sup>6</sup>

The presumed mechanism of renal AC with VA is complex and involves several pathways in different cell types.<sup>7</sup> In renal tubular cells, VA exposure will lead to translocation of phosphatidylserine (PS) to the outer leaflet of the plasma membrane. This externalization of PS inflicts release of transforming growth factor- $\beta$  (TGF- $\beta$ ) in neighbouring cells via ligation of PS receptors. Binding of TGF- $\beta$  to the TGF- $\beta$  receptor results in increased expression of CD-73 via nuclear translocation of transcription factor mothers against decapentaplegic homolog 3 (SMAD-3). This increased CD-73 expression increases adenosine formation. Activation of adenosine receptor (AR) then results in sphingosine kinase (SK-1) upregulation directly via hypoxic inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) signalling or indirectly via increased interleukin (IL)-11 synthesis by activation of extracellular regulated kinase/mitogen-activated protein kinase (ERK/MAPK). SK-1 itself promotes sphingosine-1-phosphate (S1P) synthesis. Sphingosine-1-phosphate signalling is associated with cell survival and cell growth by activation of the S1P receptor (S1PR). Furthermore, in the immune system S1P is a regulator of T- and B-cell trafficking and is directly able to suppress the Toll-like receptor (TLR)-mediated immune response from T cells.<sup>7</sup>

Experiments on pulmonary epithelial and endothelial cells suggest that the trifluorinated carbon groups of VA are responsible for the anti-inflammatory and immunomodulatory effects.<sup>8</sup>

To date, the choice of anaesthetic agent in renal transplantation is mainly based on the individual preference of the attending anaesthetist or based on local institutional protocols. Given that IRI is inevitable in organ transplantation and AC might be an effective way to reduce IRI, we designed the Volatile

Anaesthetic Protection Of Renal transplants (VAPOR) trial, which is a two-step study looking at the effect of two commonly used anaesthetic agents on renal outcome in kidney transplantation. As the first step, we report here the results of the VAPOR-1 trial, a pilot study in which propofol-based anaesthesia was compared with sevoflurane-based anaesthesia in living donor kidney transplantation (LDKT). We have chosen LDKT for the first step because it is a homogeneous and reproducible model of kidney transplantation. It provided us with a maximally controllable research setting, with optimal kidneys and similar ischaemia times. Given that the rate of failure defined as delayed graft function (DGF) is low (<5%) compared with renal transplantation with kidneys from deceased brain death donor (15–40%) or deceased circulatory death donor (40–80%), we considered VAPOR-1 a proof of concept.

We hypothesized that sevoflurane-based anaesthesia is able to induce renal AC and thereby reduces post-transplant renal injury reflected by lower concentrations of kidney injury biomarkers compared with propofol-based anaesthesia.

**Methods****Study design and population**

This prospective, randomized controlled pilot project was conducted at the University Medical Centre Groningen between September 2010 and October 2014. The Institutional Review Board approved the study protocol (METc 2009/334), which was conducted in adherence to the Declaration of Helsinki, and registered with ClinicalTrials.gov: NCT01248871. Inclusion criteria were as follows: age  $\geq 18$  yr, donation of the left kidney, and written informed consent. Exclusion criteria were as follows: ABO-incompatible transplantation, altruistic donors, and BMI  $\leq 17$  or  $\geq 35$  kg m<sup>-2</sup>. Only left kidneys were included because the gonadal vein was used for venous sampling upon reperfusion. The follow-up period was 2 yr.

**Sample size calculation**

Owing to the lack of available data in this investigational area, it was difficult to perform an adequate sample size calculation based on published data. However, we did perform a sample size calculation based on clinical urinary kidney injury molecule-1 (KIM-1) concentrations in living donors in our own centre (Nijboer WN, Leuvenink HGD, Ploeg RJ. University Medical Centre Groningen, unpublished data) to give us some idea of group size.

In a one-way ANOVA with suspected means of 100, 150, and 200 ng ml<sup>-1</sup> and a common SD within a group of 90 ng ml<sup>-1</sup>, we would have needed 17 patients per group (at a significance level of 0.05 and a power of 80%). Based upon this calculation, we decided to include 20 couples per group.

**Randomization**

Randomization was performed by the attending anaesthetist using sealed envelopes. Sixty donor-recipient couples (120 patients in total) were equally assigned to one of the following groups: PROP, propofol for donor and recipient, control group; SEVO, sevoflurane for donor and recipient, anaesthetic pre- and postconditioning; and PROSE, propofol for donor and sevoflurane for recipient, anaesthetic postconditioning. Owing to negative results in animal experiments, we did not include a fourth group (SEPRO, sevoflurane for donor and propofol for recipient, anaesthetic preconditioning).<sup>5</sup>

### Anaesthetic protocol

Anaesthesia was performed by two anaesthetists to reduce inter-operator variability. Anaesthetic and haemodynamic management were strictly protocolized. In PROP, anaesthesia was induced and maintained with propofol, using target-controlled infusion (pharmacokinetic–pharmacodynamic model of Schnider and colleagues).<sup>9</sup> In SEVO, anaesthesia was induced with a manually administered propofol bolus and maintained with sevoflurane. In PROSE, the donor was treated as for PROP and the recipient as for SEVO. A bispectral index (BIS) monitor was used to monitor anaesthetic depth. A value between 40 and 60 was considered adequate. In all groups, analgesia was managed with remifentanyl using a target-controlled infusion system (pharmacokinetic–pharmacodynamic model of Minto and colleagues).<sup>10</sup> The initial target effect site concentration (Cet) was set at 2 ng ml<sup>-1</sup>. Neuromuscular block was accomplished with cisatracurium 0.2 mg kg<sup>-1</sup>. In donors, arterial blood pressure was monitored using a radial artery catheter. In recipients, advanced haemodynamic monitoring with PiCCO® (PULSION Medical Systems SE, Feldkirchen, Germany) was performed. The goal was to maintain a mean arterial pressure (MAP) within 80% of patient baseline measures. When hypotension occurred, the first step was to adjust the depth of anaesthesia or analgesia. If that was insufficient or not possible, the patient received one or more boluses of ephedrine (5 mg) or phenylephrine (100 µg) (choice depending on heart rate). Fluid management in the donor encompassed 60 ml kg<sup>-1</sup> of crystalloids, whereas in the recipient it was goal directed based on stroke volume variation. The goal was set at a stroke volume variation of <10% at the moment of reperfusion. Predominantly, Ringers' lactate was used. If hyponatraemia occurred, Ringers' lactate was replaced by normal saline. Colloids were not given. Fluid administration was on a continuous basis; fluid challenges were not performed. After induction, donors received ceftazidime 1000 mg and recipients received furosemide 40 mg, basiliximab 20 mg and cefuroxime 1500 mg i.v. Mannitol 20% 200 ml was given before explanting the kidney from the donor and reperfusion in the recipient. If patients were at risk for development of postoperative nausea and vomiting (PONV), ondansetron 4 mg was given. Postoperative nausea and vomiting in the post-anaesthesia care unit (PACU) was treated with a step-up schedule of ondansetron 4 mg, dexamethasone 5 mg, and droperidol 0.625 mg. Piritramide and paracetamol were used for postoperative pain management.

### Surgical technique

Kidney donation was performed via hand-assisted laparoscopy. Thereafter, the kidney was flushed and perfused with cold University of Wisconsin solution (ViaSpan, DuPont, Wilmington, NC, USA or Belzer UW™, Bridge to life, Columbia SC, USA) and stored on ice. Transplantation was performed according to the local standardized protocol. Before implantation, a small catheter (5 Fr; Tyco Healthcare Ltd, Tullamore, Ireland) was inserted in the gonadal vein. This catheter was used for venous sampling from the transplanted kidney until 30 min post-reperfusion. An 8 or 6 Fr splint in the ureter was exteriorized as a suprapubic catheter and removed routinely on day 10.

### Immunosuppressive protocol

The immunosuppressive regimen comprised triple therapy containing prednisolone, a calcineurin inhibitor, and mofetil mycophenolate. The mofetil mycophenolate and first dose of

calcineurin inhibitor were given before surgery. After induction of anaesthesia, basiliximab 20 mg and methylprednisolone 40 mg were given. A second dose of basiliximab 20 mg was given on day 4. In the event of side-effects, ciclosporin was replaced by tacrolimus or vice versa. Azathioprine was started in the event of intolerance to mofetil mycophenolate.

### Samples

Blood and urine samples were obtained at standardized time points (Supplementary material, Table S1). In recipients, additional renal venous samples were drawn via the gonadal vein catheter. These were obtained simultaneously with systemic arterial samples at 30 s, 5, 10, and 30 min after reperfusion. Open needle biopsies from the transplanted kidney were obtained before implantation and 30 min after reperfusion. Each biopsy was divided in two for embedding in paraffin and storing in RNAlater. Sampling days 1, 2, 6, and 9 were chosen for logistic reasons.

### Study end points

The primary outcome was renal injury reflected by the kidney injury biomarkers KIM-1, N-acetyl-β-D-glucosaminidase (NAG), and heart-type fatty acid binding protein (H-FABP) in splint urine. Secondary biochemical end points were plasma markers reflecting IRI and reduction of plasma creatinine concentrations in the first 9 days. Biopsies were analysed for expression of caspase 3 (apoptosis), TLRs 2 and 4 (activation of innate immunity), heme oxygenase-1 (HO-1), heat shock protein 70 (hsp70), C3 and C5AR (complement activation), intercellular adhesion molecule-1 (ICAM-1), angiopoietin 2, and its receptor Tie2 (endothelial activation). Periodic acid–Schiff-stained biopsies were scored by a renal pathologist for signs of glomerulitis, tubulitis, tubular atrophy, acute tubular necrosis, interstitial and vascular lesions, and pre-existing damage. Acute tubular necrosis scoring was performed as described by Tavares and colleagues.<sup>11</sup> This scoring system is given in Supplementary material, Table S2. The pathology scoring system is given in Supplementary material, Table S3.

Secondary clinical end points were as follows: DGF defined as need for dialysis in the first week after transplantation; primary non-function (PNF) defined as permanent lack of function of the allograft; measured glomerular filtration rate (mGFR) at 3, 6, and 12 months with use of <sup>125</sup>I-iothalamate; length of hospital stay; postoperative complications according to the Clavien–Dindo classification;<sup>12</sup> treated and biopsy-proven acute rejection (AR); and 2 yr graft and patient survival.

### Sample measurements

Urinary KIM-1 and H-FABP concentrations were measured by duoset enzyme-linked immunosorbent assay (ELISA; R&D systems, Minneapolis, MN, USA). Urinary NAG activity was measured by a modified enzyme assay using p-nitrophenyl-N-acetyl-β-D-glucosaminide as substrate. Urinary creatinine was determined on a Roche Modular chemistry analyser (Roche Diagnostics, Indianapolis, IN, USA). Plasma concentrations of cytokines were determined by multiplex ELISA (Ebioscience, San Diego, CA, USA) and analysed using Luminex 100 equipment (Linc, St Louis, MO, USA). Plasma concentrations of IL-6, IL-8, and IL-10 were determined by human ELISA kits (Ebioscience, San Diego, CA, USA). All analyses were according to the manufacturers' instructions.

Gene expression in kidney biopsies was measured as described before.<sup>13</sup> Studied genes, primer sequences, and amplicon size are given in Supplementary material, Table S4.



## Statistical analysis

For statistical analysis, SPSS version 22 (IBM Corp, Armonk, NY, USA) and GraphPadPrism (GraphPad software, Inc, La Jolla, CA, USA) version 5.04 were used. Categorical data were analysed by  $\chi^2$  or Fisher's exact tests. Continuous data were tested for normality with the use of the Shapiro–Wilk test. Values are given as the mean (SD) or median with interquartile range (IQR). For normally distributed values, ANOVA or Student's unpaired t-tests were used. If variables were not normally distributed, the Kruskal–Wallis or Mann–Whitney U-test was applied. When differences between the three groups were significant, Bonferroni post hoc testing was performed.

For the repeated measures on IL-6, IL-8, and IL-10, linear mixed models were performed testing for possible interactions between group and time. In these analyses, we used autoregression correlation between the repeated measurements. This model has also been tested for mGFR at 3, 6, and 12 months. Regarding differences between IL concentrations in renal and systemic blood samples, an area under the curve was calculated between 30 s and 30 min after reperfusion. The Wilcoxon signed-rank test was applied to test the differences between the renal and systemic samples. Differences between cold storage and reperfusion biopsies were tested with Student's paired t-tests in the event of normally distributed differences or the Wilcoxon matched-pairs signed-rank test otherwise. The Kaplan–Meier method was used to analyse acute rejection episodes. Differences between the curves were determined with the log-rank test. All reported P-values are two sided. A P-value of  $\leq 0.05$  was considered significant. The attending anaesthetist was aware of the allocation. Patients, surgeons, nephrologists, the pathologist, and laboratory analysts were blinded to treatment allocation.

## Results

From September 2010 until October 2012 (primary study completion date), 125 living donor kidney transplantations were performed in the University Medical Centre Groningen, of which 88 involved donation of the left kidney. Of those 88, four donors were altruistic donors, seven couples were ABO incompatible, two recipients had a BMI  $<17.5$  or  $>35.7$  kg m $^{-2}$ , seven patients did not give informed consent, and eight couples could not participate for logistic reasons (e.g. two transplantations on the same day) or because of participation in another study. Therefore, 60 couples were equally randomized to three groups. In PROP, two couples were excluded because of surgical protocol violations. Owing to surgical difficulties in the recipients, these kidneys were exposed to extensively prolonged and additional ischaemic episodes. In SEVO, one couple was excluded because of violation of the immunosuppressive protocol, because after surgery the recipient did not take any immunosuppressant for several days. Therefore, 57 couples were eligible for sample analysis. One couple in PROP was lost to follow-up because the recipient suffered a cardiac arrest on day 9. Resuscitation was started but was unsuccessful. Post-mortem examination showed a retroperitoneal haematoma and pulmonary aspiration. In total, 56 couples were eligible for follow-up (Fig. 1, CONSORT diagram).

## Patients

Table 1 summarizes the characteristics of donors and recipients. There were no differences between the three groups with regard to relevant baseline characteristics.

Donors were all relatively healthy persons. The most common co-morbidities were hypertension and hypercholesterolaemia.

We have combined these two in cardiovascular co-morbidity in Table 1. None of the donors was suffering from diabetes. Medications used were predominantly antihypertensive medications, such as  $\beta$ -blockers, calcium channel blockers, diuretics, angiotensin converting enzyme inhibitor or angiotensin II receptor antagonist, and statins. There were no differences between groups in the use of these medications. Most recipients had multiple co-morbidities and co-medications. Underlying kidney disease and cardiovascular co-morbidity are listed in Table 1. With regard to cardiovascular medications, the groups were comparable.

## Intraoperative parameters and anaesthesia

Clinically relevant intra- and postoperative parameters are summarized in Table 2. The duration of the procedures and warm and cold ischaemia times were identical. Patients anaesthetized with sevoflurane showed a higher average BIS value during the procedure. In recipients, MAP, also reported as average MAP during the procedure, was higher in PROP compared with SEVO and PROSE. Haemodynamic profiles over time of MAP and stroke volume variation are listed in Supplementary material, Figs S1–S3. These profiles are comparable between groups. Patients anaesthetized with sevoflurane more frequently received a bolus of ephedrine compared with patients anaesthetized with propofol. This occurred predominantly after induction of anaesthesia. No extended hypotensive periods were observed, and none of the patients received vasoactive medication on a continuous basis. In all patients, remifentanyl was started at 2 ng ml $^{-1}$  Cet. In PROP recipients, average Cet of remifentanyl during the procedure was higher compared with SEVO and PROSE.

The intraoperative amount of fluid was comparable between groups. Predominantly Ringer's lactate was used. This was replaced by one or two bags of saline (500–1000 ml) in the event of hyponatraemia; this was required in eight donors (four PROP, two SEVO, and two PROSE) and 19 recipients (seven PROP, six SEVO, and six PROSE). No colloids were used. Urine production in recipients occurred in all patients immediately upon reperfusion.

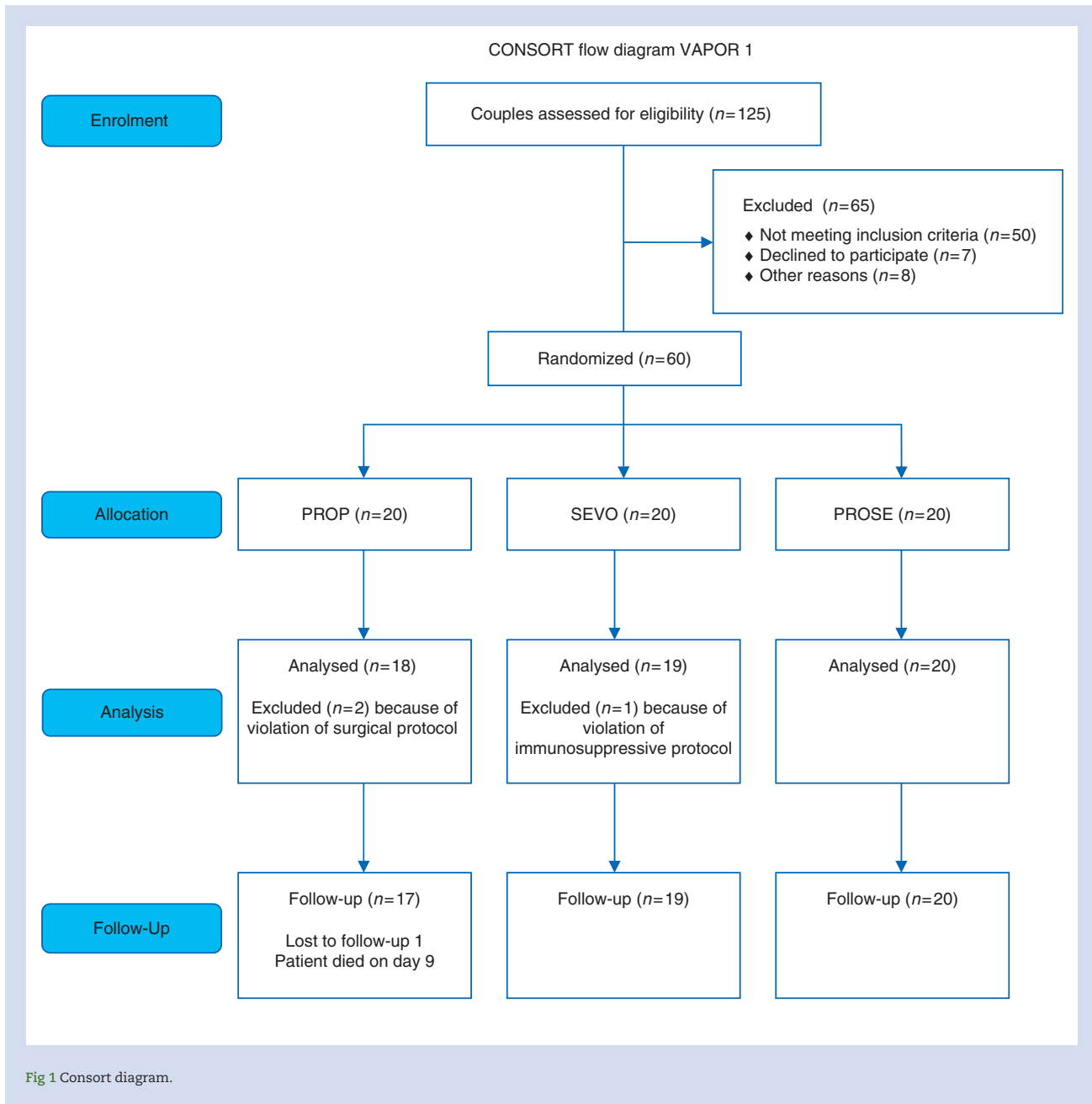
Donors in SEVO more frequently received a prophylactic dose of ondansetron. In recipients, the incidence of PONV at the PACU was significantly higher in SEVO and PROSE groups, and these patients were treated with ondansetron more frequently. On the ward, there was no difference in the incidence of nausea between groups.

## Immunosuppressants

The immunosuppressive regimens on day 7 and at 3, 6, 12, and 24 months after transplantation are listed in Supplementary material, Table S5. There were no differences between groups in the types of immunosuppressants or the trough concentrations of ciclosporin and tacrolimus at the different time points.

## Urinary renal injury markers

The KIM-1, NAG, and H-FABP concentrations were measured in splint urine (urine from the transplanted kidney) and corrected for urinary creatinine concentrations to correct for volume dilution. Sample points were as follows: first urine produced upon reperfusion, 2 h before surgery, and day 1, 2, 6 and 9 (Table S1 R6–R11). Individual concentrations per patient at different time points are displayed per group in Fig. 2. Median (IQR) concentrations and per time point are listed in Table 3. Concentrations of biomarkers in the first urine produced upon reperfusion were comparable between groups. On day 2, KIM-1 concentrations



were higher in SEVO compared with PROP ( $P=0.033$ ). PROSE showed a tendency to a higher concentration of KIM-1 on day 2 compared with PROP ( $P=0.071$ ). On day 1, NAG activity was lower in SEVO compared with PROP ( $P=0.014$ ) and PROSE ( $P=0.008$ ). On day 2, NAG activity was higher in SEVO compared with PROP ( $P=0.018$ ). Other time points and concentrations of H-FABP showed no differences (Fig. 2).

### Blood biomarkers

Cytokines were measured in plasma of the recipient. Time points were as follows: induction of anaesthesia, start of surgery, 30 s, 5, 10, and 30 min after reperfusion, 2 h postoperative, and day 1 and 2. During reperfusion, venous renal and

systemic arterial samples were obtained simultaneously (30 s, 5, 10 and 30 min after reperfusion). Concentrations of IL-1 $\beta$ , IL-4, IL-5, IL-9, IL-18, TNF- $\alpha$ , TGF- $\beta$ , and interferon- $\gamma$  were below the detection thresholds of our assays. Concentrations of IL-6, IL-8, and IL-10 were measured. The change in plasma concentrations over time did not differ between groups. Peak concentrations of IL-8 were reached 30 min post-reperfusion and of IL-6 and IL-10, respectively, 2 and 24 h after surgery. Release of IL-6 from the kidney during reperfusion was significantly higher than systemic concentrations of IL-6 ( $P<0.001$ ). This was the found in all groups. Interleukin-8 and IL-10 showed no differences in renal and systemic concentrations ( $P=0.845$  and  $P=0.226$ , respectively; Supplementary material, Fig. S4).

**Table 1** Baseline characteristics of donors and recipients. Groups are as follows: PROP (donor and recipient propofol), SEVO (donor and recipient sevoflurane), and PROSE (donor propofol and recipient sevoflurane). Data are given as the mean (sd), median (interquartile range), or n (%). HLA, human leucocyte antigen; MAP, mean arterial pressure; mGFR, glomerular filtration rate measured by isotope  $^{125}\text{I}$ -iothalamate; PRA panel specific antibodies  $\geq 15\%$

	PROP	SEVO	PROSE
<b>Donors</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>
Age (yr)	54 (19–64)	54 (38–76)	52 (38–69)
Male [n (%)]	8 (44)	9 (47)	9 (45)
BMI ( $\text{kg m}^{-2}$ )	26.1 (3.7)	27.4 (3.3)	27.1 (2.6)
ASA I/II	11/7	12/7	11/9
mGFR ( $\text{ml min}^{-1}$ )	113 (21)	116 (25)	119 (23)
Smoking [n (%)]	5 (27)	5 (26)	6 (30)
Cardiovascular co-morbidity [n (%)]	4 (22)	6 (32)	6 (30)
MAP baseline (mm Hg)	94 (93–105)	95 (85–105)	95 (86–103)
<b>Recipients</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>
Age (yr)	48.8 (15.4)	52.0 (11.5)	51.5 (10.4)
Male [n (%)]	11 (61)	8 (42)	8 (40)
BMI ( $\text{kg m}^{-2}$ )	26.1 (3.2)	25.2 (3.8)	24.8 (3.7)
ASA II/III	7/11	4/15	6/14
Underlying renal disease (n)			
Diabetes	1	2	2
IgA nephropathy	3	0	3
Autoimmune	3	1	0
Glomerulonephritis	1	0	3
Vasculitis	0	1	2
Polycystic kidney disease	1	5	3
Renal atrophy	2	4	0
Sclerosis	3	2	2
Tubulointerstitial nephritis	1	1	3
Other	3	3	2
Cardiovascular co-morbidity [n (%)]	17 (94)	19 (100)	19 (95)
MAP baseline (mm Hg)	106 (11.1)	100 (15.3)	101 (11.5)
Unrelated donor [n (%)]	9 (50)	9 (47)	11 (55)
Pre-emptive transplantation [n (%)]	9 (50)	9 (47)	10 (50)
Retransplantation [n (%)]	1 (6)	2 (10)	4 (20)
$\geq 3$ HLA mismatches [n (%)]	8 (44)	13 (68)	15 (75)
Positive PRA [n (%)]	1 (6)	2 (11)	4 (20)

### Biopsy analysis

Gene expression in cold storage and reperfusion biopsies are listed in Supplementary material, Table S6. Regarding the cold biopsies, two groups were compared (PROP-PROSE vs SEVO) because at that moment the kidney was exposed to either propofol or sevoflurane. There were no differences in gene expressions with the exception of HO-1, which was higher in SEVO. There were no differences in reperfusion biopsies between groups. Hsp70 was upregulated in reperfusion biopsies in all groups, and Tie2 was downregulated in PROP. Pathology scores are listed in Supplementary material, Table S7. No signs of pre-existing damage were observed, and biopsy scores did not differ between groups. All biopsies showed signs of acute tubular necrosis assessed by cytoplasmic changes or apoptosis of the tubular epithelium. In cold storage biopsies, coalescent groups of necrotic tubules were seen in the renal cortex. There were more extensive areas of tubular necrosis in 66% of the PROSE, 70% of the SEVO, and 90% of the PROP reperfusion biopsies.

Medullary biopsies were excluded.

### Creatinine reduction

Relative creatinine reduction from baseline for the first 9 days is displayed in Supplementary material, Table S8. The baseline was the creatinine concentration on the morning of transplantation. There were no differences between groups during the first 8 days. On day 9, SEVO showed a greater reduction compared with PROP ( $P=0.047$ ).

### Clinical end points

Clinical end points are listed in Table 4. Two patients in PROP and one in SEVO experienced DGF. Two grafts were lost because of rejection. There was no difference between groups in mGFR at 3, 6, and 12 months. Over time there was a similar decline in GFR, with an average decline of  $0.6 \text{ ml min}^{-1} \text{ month}^{-1}$  ( $P<0.001$ ).

Postoperative complications were comparable between groups. One patient in PROP died on day 9 as described in the Methods.

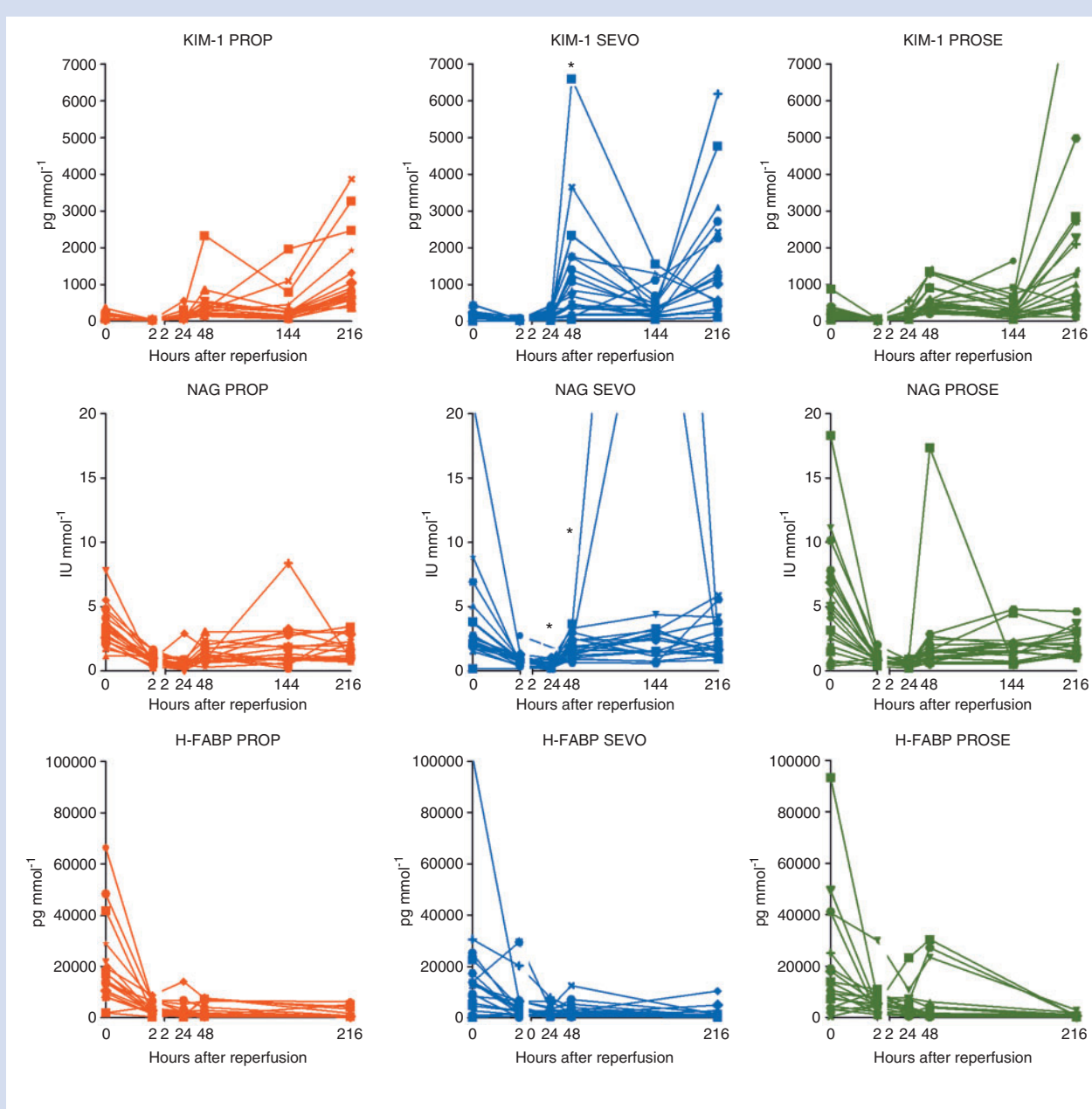
### Acute rejection

During a 2 yr follow-up, nine of 56 patients (16%) experienced acute rejection (Table 4). All rejections were T-cell mediated, and donor-

**Table 2** Intra- and postoperative parameters. Groups are as follows: PROP (donor and recipient propofol), SEVO (donor and recipient sevoflurane), and PROSE (donor propofol and recipient sevoflurane). Data are given as the mean (SD), median (interquartile range), or n (%). Continuous data were tested with ANOVA or the Kruskal–Wallis test (three groups) or Student’s unpaired t-test or the Mann–Whitney U-test (two groups). Categorical data were analysed with Fisher’s exact test. BIS, bispectral index; BW, body weight; Cet, effect site concentration; MAP, mean arterial pressure; PACU, postanesthesia care unit; PaO<sub>2</sub>, arterial partial pressure of oxygen; PONV, postoperative nausea and vomiting. Warm ischaemia time 1 was defined as the time between division of the renal artery and cold perfusion with University of Wisconsin solution; cold ischaemia time was defined as the total cold storage time; and warm ischaemia time 2 was defined as the time between cold storage and recirculation (anastomosis time)

	PROP	SEVO	PROSE	P-value
<b>Donor</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>	
Duration of procedure (min)	232 (32)	243 (41)	239 (33)	0.626
Perioperative fluid [ml (kg BW) <sup>-1</sup> ]	59.8 (12.3)	60.0 (11.1)	60.1 (11.3)	0.996
BIS	38 (7)	45 (6)	40 (4)	0.001
MAP (mm Hg)	87 (9)	75 (17)	85 (16)	0.066
Blood sample clip renal artery				
pH	7.41 (0.03)	7.39 (0.04)	7.39 (0.04)	0.230
PaO <sub>2</sub> (kPa)	19.1 (4.6)	19.8 (4.8)	19.0 (5.2)	0.876
Haemoglobin (mmol litre <sup>-1</sup> )	7.3 (0.9)	7.3 (1.0)	7.1 (0.8)	0.628
Lactate (mmol litre <sup>-1</sup> )	1.5 (0.4)	1.7 (0.7)	1.6 (0.7)	0.432
Medication				
Propofol Cet	3.3 (0.5)	—	3.1 (0.4)	0.301
Sevoflurane End tidal concentration	—	1.53 (0.14)	—	—
Remifentanyl Cet (ng ml <sup>-1</sup> )	2.9 (0.9)	2.6 (0.8)	2.8 (1.0)	0.583
Vasoactive medication				
Ephedrine [n (%)]	12 (67)	19 (100)	14 (70)	0.011
Dose (mg)	13 (6)	19 (11)	17 (8)	0.240
Phenylephrine [n (%)]	4 (22)	1 (5)	1 (5)	0.260
Dose (μg)	200 (125–200)	200 (100)	300 (300)	0.179
Piritramide				
intraoperative (mg)	7.8 (1.3)	7.9 (1.1)	7.9 (1.2)	0.958
PACU (mg)	14.0 (10–18.5)	12.0 (9–21)	12.0 (9.3–14.8)	0.549
Ondansetron intraoperative [n (%)]	1 (6%)	8 (42%)	2 (10%)	0.015
PONV [n (%)]				
PACU	5 (28)	4 (21)	9 (45)	0.270
Ondansetron	4 (22)	3 (16)	7 (35)	0.230
Dexamethasone	1 (6)	3 (16)	2 (10)	0.766
Droperidol	1 (6)	1 (5)	1 (5)	1.000
<b>Recipient</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>	
Duration of procedure (min)	200 (29)	217 (33)	202 (27)	0.156
intraoperative fluid [ml (kg BW) <sup>-1</sup> ]	55.9 (13.0)	58.2 (17.8)	64.8 (9.6)	0.127
Average BIS	38 (7)	46 (7)	47 (4)	<0.001
Average MAP (mm Hg)	92 (12)	83 (8)	80 (7)	0.001
Blood sample reperfusion				
pH	7.38 (0.04)	7.37 (0.05)	7.37 (0.08)	0.657
PaO <sub>2</sub> (kPa)	17.2 (5.8)	17.5 (4.4)	16.8 (5.6)	0.915
Haemoglobin (mmol litre <sup>-1</sup> )	5.6 (5.1–6.3)	5.4 (4.8–6.1)	5.7 (4.9–6.0)	0.737
Lactate (mmol litre <sup>-1</sup> )	1.4 (0.4)	1.7 (0.6)	1.6 (0.5)	0.305
Medication				
Propofol Cet	3.3 (0.6)	—	—	—
Sevoflurane End tidal concentration	—	1.39 (0.27)	1.43 (0.18)	0.611
Remifentanyl Cet (ng ml <sup>-1</sup> )	3.3 (0.9)	2.4 (0.7)	2.5 (0.7)	0.001
Vasoactive medication bolus				
Ephedrine [n (%)]	5 (28)	18 (95)	15 (75)	<0.001
Dose (mg)	10 (7.5–10)	15 (10–25)	15 (10–30)	0.083
Phenylephrine [n (%)]	3 (17)	4 (21)	2 (10)	0.312
Dose (μg)	200 (200)	300 (100–1300)	225 (150–300)	0.815
Piritramide (mg)				
intraoperative (mg)	8.0 (7.0–8.3)	7.0 (7.0–8.0)	7.0 (6.3–8.0)	0.259
PACU (mg)	15.4 (5.7)	15.9 (7.2)	15.5 (8.3)	0.975
Ondansetron perioperative [n (%)]	1 (6)	6 (32)	4 (20)	0.147
PONV [n (%)]				
PACU	0 (0)	4 (21)	9 (45)	0.003
Ondansetron PACU	0 (0)	3 (16)	8 (40)	0.004
Dexamethasone PACU	0 (0)	1 (5)	2 (10)	0.310
Droperidol PACU	0 (0)	3 (16)	1 (5)	0.643
Ward	7 (39)	4 (21)	6 (30)	0.546
<b>Kidney</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>	
Ischaemia time (min)				
Warm ischaemia time 1	4 (3–4)	4 (3–4)	4 (3–5)	0.577
Cold ischaemia time	170 (35)	175 (47)	168 (28)	0.794
Warm ischaemia time 2	41 (7.5)	45 (8.2)	42 (6.0)	0.294





**Fig 2** Urinary concentrations of renal injury markers. Time points are as follows: first urine produced upon reperfusion, 2 h post-transplantation, day 1, 2, 6, and 9. Values are corrected for creatinine and displayed per group for every individual patient. Groups are as follows: PROP (donor and recipient propofol), SEVO (donor and recipient sevoflurane), and PROSE (donor propofol and recipient sevoflurane). H-FABP, heart-type fatty acid binding protein; KIM-1, kidney injury molecule-1; NAG, N-acetyl- $\beta$ -D-glucosaminidase. Differences were tested with the Kruskal-Wallis test and *post hoc* with the Bonferroni test.

specific antibodies were negative. Four rejections were cellular (BANFF classification IA, PROP three and PROSE one) and five vascular (BANFF classification IIa, PROP three and SEVO two). Figure 3 shows Kaplan-Meier curves of the occurrence of acute rejection during 2 yr follow-up. There was a difference in death-censored acute rejection between groups ( $P=0.039$ ). *Post hoc* testing revealed a difference between PROP and PROSE ( $P=0.020$ ). The difference between PROP and SEVO (11%) was not significant ( $P=0.110$ ).

## Discussion

VAPOR-1 is the first randomized clinical trial to evaluate the long-term effects of anaesthetic agents on biochemical and clinical outcomes after LDKT. The main focus was the concentration of three specific urinary renal injury markers reflecting tubular damage as a result of IRI and preservation. Although we hypothesized that sevoflurane-based anaesthesia would reduce

**Table 3** Urinary concentrations of kidney injury markers after transplantation. Groups are as follows: PROP (donor and recipient propofol), SEVO (donor and recipient sevoflurane), and PROSE (donor propofol and recipient sevoflurane). Data are given as the median (interquartile range). Data were tested with the Kruskal–Wallis test and *post hoc* with the Bonferroni test. H-FABP, heart-type fatty acid binding protein; KIM-1, kidney injury molecule-1; NAG, N-acetyl- $\beta$ -D-glucosaminidase

	PROP	SEVO	PROSE	P-value
<b>KIM-1 (pg mmol<sup>-1</sup>)</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>	
0 h	149.6 (70.0–200.0)	123.6 (81.1–207.3)	144.3 (44.8–263.0)	0.961
2 h	28.7 (19.4–34.7)	29.6 (19.0–44.5)	33.5 (24.3–46.0)	0.311
Day 1	109.2 (71.6–165.5)	138.0 (80.1–289.0)	131.2 (73.7–238.6)	0.559
Day 2	301.2 (202.0–504.7)	952.8 (311.8–1893.0)	483.6 (281.4–905.5)	0.042
				PROP–SEVO: 0.032
				PROP–PROSE: 0.071
				SEVO–PROSE: 0.176
Day 6	238.1 (131.3–347.9)	311.2 (161.8–557.9)	252.4 (153.7–423.9)	0.960
Day 9	800.6 (604.4–1618.0)	1169.0 (421.1–2577.0)	886.4 (372.0–2381.0)	0.447
<b>NAG (IU mmol<sup>-1</sup>)</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>	
0 h	3.312 (2.378–4.256)	2.61 (1.968–4.140)	4.664 (1.953–7.304)	0.389
2 h	0.899 (0.579–1.057)	0.847 (0.522–0.947)	0.838 (0.630–1.019)	0.790
Day 1	0.541 (0.384–0.768)	0.293 (0.252–0.501)	0.534 (0.464–0.775)	0.012
				PROP–SEVO: 0.014
				PROP–PROSE: 0.892
				SEVO–PROSE: 0.008
Day 2	1.078 (0.819–1.713)	1.835 (1.162–2.457)	1.502 (1.025–2.045)	0.0550
				PROP–SEVO: 0.018
				PROP–PROSE: 0.176
				SEVO–PROSE: 0.281
Day 6	1.217 (0.839–2.781)	1.991 (1.321–3.194)	1.612 (1.238–2.143)	0.309
Day 9	1.494 (0.873–2.578)	2.042 (1.286–4.016)	1.773 (1.229–2.983)	0.163
<b>H-FABP (pg mmol<sup>-1</sup>)</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>	
0 h	14 560 (9349–23 587)	12 924 (4137–18 610)	10 507 (4525–25 068)	0.433
2 h	2528 (1626–6483)	2854 (1217–6585)	4626 (2169–7878)	0.185
Day 1	1534 (864–2780)	1562 (703–3384)	2725 (1271–6117)	0.809
Day 2	838 (583–3216)	1144 (584–3043)	1063 (846–2736)	0.218
Day 9	372 (299–2539)	422 (267–1573)	470 (244–968)	0.889

IRI, reflected by reduced concentrations of these markers, concentrations of KIM-1 and NAG showed unexpected patterns.

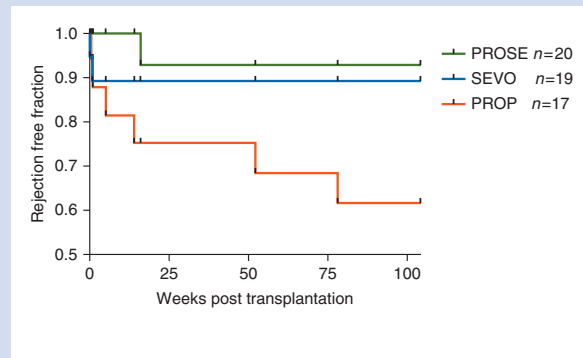
Kidney injury molecule-1, a type 1 cell membrane protein, is not expressed in healthy individuals but is markedly upregulated in chronic or acute kidney injury in proximal tubular cells, turning these cells into phagocytes.<sup>14</sup> Its ectodomain is cleaved and shed in urine, where it remains highly stable. N-Acetyl- $\beta$ -D-glucosaminidase, a lysosomal enzyme in proximal tubular cells, is rapidly released in urine upon injury. However, an increased urinary activity of this enzyme might also reflect increased lysosomal activity in renal tubular cells rather than damage to these cells. Increased urinary NAG activity is also reported in a variety of diseases, including hypertension.<sup>15</sup> Clinical performance of these specific biomarkers is an area in evolution. When this trial (2009–2010) was designed, little was known about the performance of these biomarkers in a renal transplant setting. One study by Zhang and colleagues,<sup>16</sup> measuring the expression of KIM-1 in renal transplant biopsies, showed that positive KIM-1 staining identified proximal tubular injury and that its expression was correlated with the degree of renal dysfunction. However, most studies testing the performance of these markers were related to acute kidney injury (AKI). In this setting, urinary KIM-1 and NAG have been shown to be sensitive and early diagnostic indicators of renal injury. In discriminating patients with AKI from healthy individuals, KIM-1 and NAG

showed an area under the receiver operating characteristic curve of 0.95 and 1.00, respectively. In discriminating AKI from non-AKI patients (intensive care unit patients, cardiac catheterization), this was reduced to 0.93 and 0.83, respectively.<sup>17</sup> However, the prognostic performance of these biomarkers (and many others) in predicting AKI varied greatly among studies, ranging from very negative to very positive.<sup>18</sup> We wanted to look at the entire tubular system; therefore, we have added H-FABP as a third biomarker. This cytoplasmatic protein involved in fatty acid metabolism is the least renal specific of our markers and predominantly present in myocardial cells. In the kidney, it is located in the distal tubular cells and released upon ischaemia.

In recent years, more research has been performed on the role of urinary biomarkers and their ability to predict (long-term) graft outcome in renal transplantation. Concentrations of KIM-1 were measured in the urine of donors and recipients and in machine perfusate of kidneys from living and deceased donors.<sup>19–21</sup> The results range from no prognostic performance at all to poor prediction of DGF post-transplantation. In most of these studies, KIM-1 is outperformed by other injury markers, such as neutrophil gelatinase-associated lipocalin. It has been suggested by several authors that increased KIM-1 concentrations might indicate on-going recovery and regeneration after injury. This may lead to a paradigm shift in thinking about biomarkers from substances released upon injury in which the

**Table 4** Clinical outcomes in recipients. Groups are as follows: PROP (donor and recipient propofol), SEVO (donor and recipient sevoflurane), and PROSE (donor propofol and recipient sevoflurane). Data are given as the median (interquartile range) or *n* (%). mGFR, measured glomerular filtration rate measured with  $^{125}\text{I}$ -iothalamate; mGFR was tested with linear mixed models, with autoregression correlation between repeated measurements. Continuous data were analysed with the Kruskal–Wallis test. Categorical data were analysed with Fisher's exact test. Acute rejection episodes were tested with the log-rank test

	PROP	SEVO	PROSE	P-value
<b>Renal function</b>	<b>n=18</b>	<b>n=19</b>	<b>n=20</b>	
Urinary splint output during first 2 h (ml)				
First hour	325 (150–350)	275 (170–350)	303 (156–350)	0.868
Second hour	350 (194–644)	350 (145–500)	275 (175–444)	0.492
mGFR (ml min <sup>-1</sup> )				
3 months	64 (51–68) (n=13)	66 (56–76) (n=17)	60 (49–71) (n=19)	0.505
6 months	61 (50–71) (n=16)	68 (57–78) (n=18)	56 (47–71) (n=17)	
1 yr	57 (48–65) (n=19)	59 (46–67) (n=19)	54 (44–67) (n=19)	
Delayed graft function [n (%)]	2 (11)	1 (5)	0 (0)	0.199
Primary non-function [n (%)]	0 (0)	0 (0)	0 (0)	1.000
Graft loss [n (%)]	n=17 1 (6)	n=19 1 (5)	n=20 0 (0)	0.536
Acute rejection 2 yr [n (%)]	6/17 (35)	2/19 (11)	1/20 (5)	0.039
<b>Postoperative course</b>				
Complications Clavien–Dindo				
Grade I	5	4	5	0.928
Grade II	3	3	3	0.670
Grade IIIa	2	0	0	0.096
Grade III	1	2	2	1.000
Grade IVa	0	0	0	1.000
Grade IV	0	0	0	1.000
Grade V	1	0	0	0.316
Hospital stay (days)	17 (17–18.25)	17 (17–17)	17 (17–17)	0.457



**Fig 3** Kaplan–Meier curves of the occurrence of acute rejection during 2 yr follow-up. The rejection-free fraction is displayed on the y-axis. Groups are as follows: PROP (donor and recipient propofol), SEVO (donor and recipient sevoflurane), and PROSE (donor propofol and recipient sevoflurane). Acute rejection episodes were tested with the log-rank test.

amount of biomarker is correlated with the amount of injury (good predictor) to a more functional role of these substances in healing and repair, making it a less strong or poor predictor of outcome. In our study, concentrations of KIM-1, NAG, and H-FABP in the first urine upon reperfusion were comparable between groups. After this, H-FABP concentrations declined

during the post-transplant period and, in most patients, did not increase again. In contrast, KIM-1 and NAG showed a different pattern. After an initial decrease in KIM-1, concentrations in our population increased again. On day 2, KIM-1 concentrations were higher in SEVO compared with PROP. PROSE showed a tendency to higher concentrations compared with PROP at this time point. *In vitro* studies showed that shedding of the ectodomain of KIM-1 in urine is mediated by activation of ERK, and this cleavage is accelerated by p38 MAPK activation.<sup>22</sup> Interestingly, in proximal tubular cells, sevoflurane treatment activates ERK and it provides neuroprotection by activation of p38 MAPK 24–72 h after reperfusion in the rat brain.<sup>23 24</sup>

As stated above, evidence is accumulating that AKI KIM-1 may play an important role in the regeneration and repair process. Recently, Yang and colleagues<sup>25</sup> are able to phagocytize luminal cellular debris consisting of apoptotic and necrotic cells, enabling the proximal tubular cell to downregulate the innate immune response upon AKI. This could be beneficial in kidney transplantation because an inflammatory environment attributable to parenchymal injury during transplantation makes the graft more prone to acute and chronic rejection.<sup>26 27</sup> Furthermore, in renal transplant recipients with AKI, Zhang and colleagues<sup>16</sup> showed that higher concentrations of KIM-1 expression were associated with a better recovery over time. In our study, the second increase at day 9 in all groups could be explained by calcineurin inhibitor nephrotoxicity.<sup>28 29</sup>

Regarding NAG in our study, the highest NAG activity was observed in the first urine produced. This is probably a true reflection of the IRI and preservation process. After a decrease

in activity over the first day, it increased again on day 2 after transplantation and generally remained stable on days 6 and 9. On day 2, SEVO showed higher concentrations compared with PROP. This could be a reflection of regenerated tubular cells showing baseline lysosomal activity. Kotanko and colleagues<sup>30</sup> showed that a low urinary NAG activity between week 2 and 4 post-transplantation is associated with poorer graft survival after 4 yr compared with high urinary NAG activity during this period.

Overall, our results can be interpreted two ways: the second increase of the biomarkers KIM-1 and NAG could be attributable to injury or (in our opinion, more likely) it could be associated with increased regeneration and recovery of the tubular system. Higher concentrations were not associated with inferior graft outcome. Concentrations of KIM-1 and NAG on day 2 were strongly correlated ( $P < 0.001$ ), and the correlation of estimated glomerular filtration rate (eGFR) at 1 month and KIM-1 concentrations at day 2 was almost significant ( $P = 0.074$ ), where a higher KIM-1 concentration was correlated with a higher eGFR.

Expression of HO-1 in cold biopsies was higher in kidneys exposed to sevoflurane compared with kidneys exposed to propofol. Sevoflurane-induced upregulation of HO-1 has been described in different cell types.<sup>31, 32</sup> Organ protection via the HO-1 pathway is probably one of the pathways involved in anaesthetic conditioning with VA. However, in reperfusion biopsies this difference was no longer visible. This can be explained by the fact that upon injury most cell types upregulate HO-1 as a mechanism of self-protection. This was also reflected in the extensive upregulation of hsp-70 in all groups upon reperfusion.

Pathology scores did not differ between groups. This could be because of the fact that our protocol biopsies were obtained ~30 min after reperfusion at skin closure. In the literature, differences are found only 3 h post-reperfusion.<sup>5</sup>

This study has several limitations. The LDKT setting provided an optimal research setting but also had substantial drawbacks. We did expect that, although the amount of injury in this setting is lower compared with injury in kidneys from deceased donors, cytokines reflecting IRI would be measurable in plasma. But to our surprise, many of these cytokines were below the detection threshold. Many factors influence conditioning strategies, including patients' co-morbidity and use of medication. We have looked at these factors, and the groups are comparable. However, we cannot exclude the possibility that co-morbidity or medication might have played a role in the success or failure of our conditioning strategies. Some minor, although statistically significant, differences between groups regarding intraoperative care were observed. Ephedrine, mostly administered after induction of anaesthesia, was given more frequently in patients anaesthetized with sevoflurane. In these patients, anaesthesia was induced with a manually administered bolus of propofol, which might induce larger cardiovascular changes compared with the target-controlled infusion-administered propofol in the propofol-based anaesthesia. Profound hypotension over extended periods was not observed, and continuous vasopressor support was not required. Although the MAP during the procedure was lower in recipients anaesthetized with sevoflurane, it was still >80% of the patients' baseline range. To stay within this range, remifentanyl Cet was adjusted, resulting in a lower Cet in recipients anaesthetized with sevoflurane. Protection against IRI with the use of remifentanyl has been described for several organs in animal experiments. Most of these studies used a conditioning strategy of two or three times a cycle of 5 min remifentanyl infusion followed by 5 min of reperfusion. The doses used in these experiments are rather high (heart,  $6.0 \mu\text{g kg}^{-1} \text{ min}^{-1}$ ) compared with the continuous dose we used

in our clinical setting (range  $0.08\text{--}0.12 \mu\text{g kg}^{-1} \text{ min}^{-1}$ ).<sup>33</sup> One study using lower doses ( $0.1\text{--}1 \mu\text{g kg}^{-1} \text{ min}^{-1}$ ) for preconditioning of the intestine reported a dose-independent effect.<sup>34</sup> Studies using continuous or semi-continuous infusion during the entire procedure or before and during the ischaemic period show a dose-independent effect in the liver (dose ranging from  $0.4$  to  $10.0 \mu\text{g kg}^{-1} \text{ min}^{-1}$ ), but in the brain a protective effect was seen only in the high range dose ( $1.8 \mu\text{g kg}^{-1} \text{ min}^{-1}$ ).<sup>35, 36</sup> In our opinion, the difference in Cet between our groups does not have any clinical significance or any effect on conditioning. The lower Cet of remifentanyl might also result in greater arousability, leading to a higher overall BIS value in SEVO and PROSE. However, the overall depth and stability of anaesthesia can be considered clinically similar among groups without inducing effects on the study objectives. Volatile anaesthetics are a known cause of PONV. Therefore, the increased use of ondansetron for PONV in sevoflurane groups was anticipated.

To our surprise, we observed a significant difference in the occurrence of T-cell-mediated rejection between groups during the first 2 yr after transplantation in favour of the sevoflurane groups. As there were only nine events, we could not perform an adequate multivariate analysis. However, known risk factors, such as human leucocyte antigen mismatches, panel specific antibodies >15%, and second or third transplantation, had a higher incidence in SEVO and PROSE. It has been shown that both anaesthetic agents have differential effects on cells of the immune system. Several studies have shown the inhibitory effects of VA on lymphocyte proliferation and cytokine release and the ability of VA to induce apoptosis in T lymphocytes. Propofol has a minor effect on lymphocyte proliferation and function.<sup>37</sup> It has also been shown *in vitro* that both propofol and sevoflurane blockage Lymphocyte function-associated antigen 1 (LFA-1) at the lovastatin binding site.<sup>38, 39</sup> Blockage of LFA-1 is recognized as a potential target to reduce allograft rejection, through effects on T-cell migration and antigen presentation.<sup>40, 41</sup> Although both anaesthetics possess this ability *in vitro*, we do not know whether this effect is comparable *in vivo*. Unfortunately, we did not collect cells and were unable to look at the effects of both agents on cell subtypes.

As the next step, we will proceed with VAPOR-2, a multicentre randomized controlled trial comparing sevoflurane-based anaesthesia vs propofol-based anaesthesia on clinical renal outcome (DGF) in kidney transplantation with kidneys of deceased donors.

In conclusion, in LDKT sevoflurane- or propofol-based anaesthesia resulted in comparable concentrations of urinary renal biomarkers in the first urine produced upon reperfusion. On day 2, sevoflurane-based anaesthesia led to higher urinary concentrations of KIM-1 and NAG but not H-FABP. These higher concentrations were not associated with inferior graft outcome. Remarkably, a lower acute rejection rate after 2 yr was seen in recipients receiving sevoflurane.

## Authors' contributions

Research design: G.J.N.M., V.B.N., M.A.J.S., R.J.P., H.G.D.L., M.M.R.F.S.  
Execution of the trial: G.J.N.M., V.B.N., M.A.J.S., R.J.P.  
Data analysis: G.J.N.M., M.C.H., J.G.M.B., P.J.O., H.G.D.L., M.M.R.F.S.  
Writing of the paper: G.J.N.M., V.B.N., M.A.J.S., S.P.B., M.C.H., J.G.M.B., P.J.O., R.J.P., H.G.D.L., M.M.R.F.S.

## Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.



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## Declaration of interest

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R.J.P.: his research group/department has received no funds from private companies. R.J.P. holds the following patents: Plaats van der A, Verkerke GJ, Rakhorst G, Ploeg RJ, Hart t NA, Leuvenink HGD. Transportable perfusion and preservation device for donor organs. Dutch patent nr. 1024022, 2003. Zuidema J, Hak JB, Smit J, Cate Hoedemaker HO, Ploeg RJ. Devices and methods for anastomosis Dutch patent nr. P68635EP00, 2004. He is also an Associate Editor of the journals *Transplantation* and *American Journal of Transplantation* and occasionally advises the companies Bridge to Life and Teva on transplant-related matters.

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## References

- Minguet G, Joris J, Lamy M. Preconditioning and protection against ischaemia-reperfusion in non-cardiac organs: a place for volatile anaesthetics? *Eur J Anaesthesiol* 2007; **24**: 733–45
- Freedman BM, Hamm DP, Everson CT, Wechsler AS, Christian CM. Enflurane enhances postischemic functional recovery in the isolated rat heart. *Anesthesiology* 1985; **62**: 29–33
- Cason BA, Gamperl AK, Slocum RE, Hickey RF. Anesthetic-induced preconditioning: previous administration of isoflurane decreases myocardial infarct size in rabbits. *Anesthesiology* 1997; **87**: 1182–90
- Symons JA, Myles PS. Myocardial protection with volatile anaesthetic agents during coronary artery bypass surgery: a meta-analysis. *Br J Anaesth* 2006; **97**: 127–36
- Lee HT, Ota-Setlik A, Fu Y, Nasr SH, Emala CW. Differential protective effects of volatile anesthetics against renal ischemia-reperfusion injury in vivo. *Anesthesiology* 2004; **101**: 1313–24
- Lee HT, Kim M, Kim M, et al. Isoflurane protects against renal ischemia and reperfusion injury and modulates leukocyte infiltration in mice. *Am J Physiol Renal Physiol* 2007; **293**: F713–22
- Fukazawa K, Lee HT. Volatile anesthetics AKI: risks, mechanisms, and a potential therapeutic window. *J Am Soc Nephrol* 2014; **25**: 884–92
- Urner M, Limbach LK, Herrmann IK, et al. Fluorinated groups mediate the immunomodulatory effects of volatile anesthetics in acute cell injury. *Am J Respir Cell Mol Biol* 2011; **45**: 617–24
- Schnider TW, Minto CF, Gambus PL, et al. The influence of method of administration and covariates on the pharmacokinetics of propofol in adult volunteers. *Anesthesiology* 1998; **88**: 1170–82
- Minto CF, Schnider TW, Shafer SL. Pharmacokinetics and pharmacodynamics of remifentanyl. II. Model application. *Anesthesiology* 1997; **86**: 24–33
- Tavares MB, Chagas de Almeida Mda C, Martins RT, de Sousa AC, Martinelli R, dos-Santos WL. Acute tubular necrosis and renal failure in patients with glomerular disease. *Ren Fail* 2012; **34**: 1252–7
- Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205–13
- Damman J, Nijboer WN, Schuurs TA, et al. Local renal complement C3 induction by donor brain death is associated with reduced renal allograft function after transplantation. *Nephrol Dial Transplant* 2011; **26**: 2345–54
- Bonventre JV, Yang L. Kidney injury molecule-1. *Curr Opin Crit Care* 2010; **16**: 556–61
- Bosomworth MP, Aparicio SR, Hay AW. Urine N-acetyl-beta-D-glucosaminidase—a marker of tubular damage? *Nephrol Dial Transplant* 1999; **14**: 620–6
- Zhang PL, Rothblum LI, Han WK, Blasick TM, Potdar S, Bonventre JV. Kidney injury molecule-1 expression in transplant biopsies is a sensitive measure of cell injury. *Kidney Int* 2008; **73**: 608–14
- Vaidya VS, Waikar SS, Ferguson MA, et al. Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. *Clin Transl Sci* 2008; **1**: 200–8
- Vanmassenhove J, Vanholder R, Nagler E, Van Biesen W. Urinary and serum biomarkers for the diagnosis of acute kidney injury: an in-depth review of the literature. *Nephrol Dial Transplant* 2013; **28**: 254–73
- Parikh CR, Hall IE, Bhargoo RS, et al. Associations of perfusate biomarkers and pump parameters with delayed graft function and deceased donor kidney allograft function. *Am J Transplant* 2016; **16**: 1526–39



20. Koo TY, Jeong JC, Lee Y, et al. Pre-transplant evaluation of donor urinary biomarkers can predict reduced graft function after deceased donor kidney transplantation. *Medicine (Baltimore)* 2016; **95**: e3076
21. Reese PP, Hall IE, Weng FL, et al. Associations between deceased-donor urine injury biomarkers and kidney transplant outcomes. *J Am Soc Nephrol* 2016; **27**: 1534–43
22. Zhang Z, Humphreys BD, Bonventre JV. Shedding of the urinary biomarker kidney injury molecule-1 (KIM-1) is regulated by MAP kinases and juxtamembrane region. *J Am Soc Nephrol* 2007; **18**: 2704–14
23. Lee HT, Kim M, Song JH, Chen SWC, Gubitosa G, Emala CW. Sevoflurane-mediated TGF- $\beta_1$  signaling in renal proximal tubule cells. *Am J Physiol Renal Physiol* 2008; **294**: F371–8
24. Ye Z, Guo Q, Wang N, Xia P, Yuan Y, Wang E. Delayed neuroprotection induced by sevoflurane via opening mitochondrial ATP-sensitive potassium channels and p38 MAPK phosphorylation. *Neuro Sci* 2012; **33**: 239–49
25. Yang L, Brooks CR, Xiao S, et al. KIM-1-mediated phagocytosis reduces acute injury to the kidney. *J Clin Invest* 2015; **125**: 1620–36
26. Halloran PF, Melk A, Barth C. Rethinking chronic allograft nephropathy: the concept of accelerated senescence. *J Am Soc Nephrol* 1999; **10**: 167–81
27. Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994; **12**: 991–1045
28. Carlos CP, Sonehara NM, Olini SM, Burdmann EA. Predictive usefulness of urinary biomarkers for the identification of cyclosporine A-induced nephrotoxicity in a rat model. *PLoS One* 2014; **9**: e103660
29. Cosner D, Zeng X, Zhang PL. Proximal tubular injury in medullary rays is an early sign of acute tacrolimus nephrotoxicity. *J Transplant* 2015; **2015**: 142521
30. Kotanko P, Margreiter R, Pfaller W. Reduced renal allograft survival is related to low urinary N-acetyl- $\beta$ -D-glucosaminidase excretion during the first posttransplant month. *Transplantation* 1996; **61**: 388–92
31. Hoetzel A, Leitz D, Schmidt R, et al. Mechanism of hepatic heme oxygenase-1 induction by isoflurane. *Anesthesiology* 2006; **104**: 101–9
32. Xiong XQ, Lin LN, Wang LR, Jin LD. Sevoflurane attenuates pulmonary inflammation and ventilator-induced lung injury by upregulation of HO-1 mRNA expression in mice. *Int J Nanomedicine* 2013; **6**: 1075–81
33. Qiao S, Mao X, Wang Y, et al. Remifentanyl preconditioning reduces postischemic myocardial infarction and improves left ventricular performance via activation of the janus activated kinase-2/signal transducers and activators of transcription-3 signal pathway and subsequent inhibition of glycogen synthase kinase-3 $\beta$  in rats. *Crit Care Med* 2016; **44**: e131–45
34. Shen JT1, Li YS1, Xia ZQ1, et al. Remifentanyl preconditioning protects the small intestine against ischemia/reperfusion injury via intestinal  $\delta$ - and  $\mu$ -opioid receptors. *Surgery* 2016; **159**: 548–59
35. Liu X, Pan Z, Su D, et al. Remifentanyl ameliorates liver ischemia-reperfusion injury through inhibition of interleukin-18 signaling. *Transplantation* 2015; **99**: 2109–17
36. Zhang TZ1, Zhou J, Jin Q, et al. Protective effects of remifentanyl preconditioning on cerebral injury during pump-assisted coronary artery bypass graft. *Genet Mol Res* 2014; **13**: 7658–65
37. Kurosawa S, Kato M. Anaesthetics, immune cells, and immune responses. *J Anesth* 2008; **22**: 263–77
38. Yuki K, Astrof NS, Bracken C, Soriano SG, Shimaoka M. Sevoflurane binds and allosterically blocks integrin lymphocyte function-associated antigen-1. *Anesthesiology* 2010; **113**: 600–9
39. Yuki K, Bu W, Xi J, Shimaoka M, Eckenhooff R. Propofol shares the binding site with isoflurane and sevoflurane on leukocyte function-associated antigen-1. *Anesth Analg* 2013; **117**: 803–11
40. Badell IR, Russell MC, Thompson PW, et al. LFA-1-specific therapy prolongs allograft survival in rhesus macaques. *J Clin Invest* 2010; **120**: 4520–31
41. Lunsford KE, Barbas AS, Brennan TV. Recent advances in immunosuppressive therapy for prevention of renal allograft rejection. *Curr Opin Organ Transplant* 2011; **16**: 390–7

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